Chimeric antigen receptors (CARs) are artificial molecules that can be used to redirect T cell immune response against antigens expressed on the surface of tumor cells. Although promising, most current protocols expand engineered T cells non-specifically using IL-2 and OKT3, which decreases the frequency of transgenic populations over time. Additionally, cell expansion using conventional cultureware is complicated and labor intensive, which limits the broader application of this therapy. With the grant support from Production Assistance for Cell Therapy (PACT), we assessed whether CAR T cell manufacture could be optimized and streamlined by: (i) supplementing non-specific stimuli (IL-2) with an artificial antigen presenting cell (a-APC) engineered to express cognate antigen and co-stimulatory molecules, and (ii) efficiently and rapidly expanding cells in a simple and scalable gas permeable culture device (G-Rex). As a proof of principle, we sought to expand T cells engineered with a CAR targeting the prostate cancer antigen, PSCA. We first generated an a-APC cell line by modifying K562 cells, which expressed a range of co-stimulatory molecules including CD80, CD86, and 41BBL, with a retroviral vector encoding the PSCA antigen. After the co-culture of CAR-PSCA T cells with the irradiated a-APC, we found that a-APCs co-expressing PSCA antigen, CD80, and 41BBL were the most effective in inducing T cell expansion, with a 1.9 fold increase in total cell numbers when compared with CAR T cells expanded in the presence of IL2 alone. We also saw an increase in the frequency of transgenic CAR T cells which increased from 36.5% to 88.1% after 10 days of culture. In contrast, the percentage of transgenic T cells was sustained when cultured in the presence of IL2 (36.5% on day 0 and 37.2% on day 10). Thus, culture of CAR-T cells with antigen-expressing a-APCs not only improves total cell output, but also enriches for transgene-expressing. Next, to assess whether we could scale up cell production we transferred the engineered a-APCs and CAR-PSCA T cells (at a 2:1 ratio) into a static GMP-compliant G-Rex with a surface area of 100cm^2. In these G-Rex devices, O_2 and CO_2 are exchanged across a silicone membrane at the base, which allows for the addition of an increased depth of medium above the cells. These culture conditions have been shown to increase cell output without increasing the number of cell doublings. From an initial seeding density of 25E+06 CAR T cells, we obtained a total of 2200-2500E+06 cells within 10 days of culture. Thus, without any intervention we obtained a 93 fold increase in cell numbers using only 1 liter of T cell culture media. We also observed an enrichment of transgenic T cells (from 33.2% to 81.7%, a 2.4±1.2 fold increase, after 10 days of culture). Taken together the total T cell fold expansion (93) and the enrichment for the transgene (2.4±1.2), we calculate a 223.5±111.6 fold expansion of CAR T cells.

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Novel Immunodominant CD4+ T Cell Epitopes in the CMV Proteins IE1 and IE2

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Background: Cytomegalovirus (CMV) continues to be an important human pathogen in allogeneic hematopoietic cell transplant (allo-HCT) recipients. Both CD4+ and CD8+ T cells are important for long-term control of the virus. Identification of CMV-specific T cell epitopes has primarily focused on CD8+ T cell epitopes. Adoptive transfer of CMV-specific CD4+ T cells has previously been associated with prolonged protection from reactivation and CMV disease.
Aims: To characterize the repertoire and restriction of CMV-specific CD4+ T cell responses against immediate early antigen 1 and 2 (IE1 and IE2) in 16 healthy, HLA-typed, CMV+ donors.
Methods: We performed IFN-γ ELISPOT assays ex vivo on PBMCs from CMV+ donors using 187 peptides (15 amino acids long and overlapping 10 amino acids) spanning the entire IE1 and IE2. These peptides were distributed into individual microtiter wells, PBMCs from each donor were added and incubated, and the resulting responses were measured by IFN-γ ELISPOT analysis. To validate the peptide-specificity and to establish the phenotype of the responding cells, peptides eliciting IFN-γ release in the ELISPOT analysis were subsequently used to stimulate PBMCs in vitro for 12-14 days followed by flow cytometric intracellular cytokine secretion assay. For the identified CD4+ T cell responses, peptide-MHC class II (MHCII) affinity measurements were used to suggest the most likely restricting element(s) among the donors MHCII molecules. To validate MHCII restriction element(s), MHCII tetramers (TMR) were generated and used to label in vitro stimulated PBMCs.
Results: We have identified 27 CD4+ T cell epitopes in IE1 and IE2; 19 of these are novel. Several CD4+ T cell epitopes appeared to be immunodominant. Thus, one DRB1*0101-restricted IE2 epitope was recognized by 5/5 DRB1*0101-positive donors, two DRB1*0301-restricted epitopes, one in IE2 and one in IE1, were recognized by 4/4 and 4/4 DRB1*0301-positive donors, respectively, and one DRB5*0101-restricted IE1 epitope was recognized by 4/4 DRB5*0101-positive donors. Additional epitopes appear to be immunodominant by functional analysis, but have not yet been validated with MHCII TMRs.
Conclusions: We have found multiple novel CD4+ epitopes in CMV IE1 and IE2 of which several have been validated with MHCII TMRs. These epitopes could be used to explore the reconstitution of CMV-specific CD4+ T cells following allo-HCT, and they could represent promising candidates for adoptive CD4+ T cell transfer.

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Evaluating Donor Lymphocyte Infusions (DLI) for the Treatment of Falling Chimerism, Minimal Residual Disease and Relapse After Pediatric Hematopoietic Stem Cell Transplantation (HSCT)

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